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几内亚格木和降香黄檀对热带北缘地区冬季低温的光合适应*

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摘要: 在热带北缘地区, 冬季气温较夏季下降 10 ℃左右, 虽然热带植物对零上低温敏感, 但是大部分热带树木能够适应热带北缘地区的冬季气温, 其光合生理机制并不清楚。我们通过测定种植在热带北缘地区(21°54′N, 101°46′E)的两种热带树木(几内亚格木和降香黄檀)的光系统 I 和 II 活性以及光系统 I 和 II 的能量分配的季节变化, 发现这两个树种的光系统 I 和 II 活性在冬季并没有下降。两个树种的光系统 II 的有效量子产额在冬季明显下降, 同时伴随着热耗散激发。在冬季, 环式电子传递的激发与热耗散的激发呈现显著的正相关。环式电子传递的激发使得氧化态 P700 比例的上升, 从而避免了光系统 I 受体端的过度还原。化学试剂抗霉素 A (PGR5 途径环式电子传递的一种特异性抑制剂) 处理过的叶片较对照组表现出更强光损伤程度。这些结果表明环式电子传递的激发是热带树木适应热带北缘地区冬季低温的一个重要的光合生理机制。

关键词: 适应; 环式电子传递; 冬季; 光系统 I; 光系统 II; 热带树木
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Photosynthetic Acclimation of *Erythrophleum guineense* and *Dalbergia odorifera* to Winter Low Temperature in a Marginal Tropical Area

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Abstract: In marginal tropical areas, air temperature in winter usually decreases by 10 ℃ compared with summer at night/day. Although tropical plants are sensitive to low temperature, the mechanism underlying photosynthetic acclimation of tropical trees to winter low temperature is unclear. To address this question, the photosystem I (PSI) and photosystem II (PSII) activities, and energy distribution in PSI and PSII were examined in summer and winter in two tropical high-quality timber tree species *Erythrophleum guineense* and *Dalbergia odorifera* grown in a marginal tropical area (21°54′N, 101°46′E). Our results indicated that the photosynthetic apparatus of *E. guineense* and *D. odorifera* was maintained stable in winter. The effective quantum yield of PSII decreased significantly in winter, but non-photochemical quenching (NPQ) significantly increased. In winter, cyclic electron flow (CEF) was significantly stimulated in both species, which was significantly and positively correlated with NPQ. Meanwhile, the stimulation of CEF led to an increase in P700 oxidation ratio and the over-reduction of PSI acceptor side was prevented.

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Antimycin A (a specific inhibitor of PGR5-dependent CEF) significantly aggravated PSII photoinhibition under high light in both species. These results suggested that stimulation of CEF is an important mechanism for photosynthetic acclimation to winter low temperature in a marginal tropical area in the two tropical tree species.

Key words: Acclimation; Cyclic electron flow; Winter; Photosystem I; Photosystem II; Tropical trees

Tropical trees species are native to tropical areas in which the air temperature in winter is usually high. Some of them produce high-quality timber. Due to the exacerbation of fragmentation of tropical forests and increasing demand for tropical hardwood timber, afforestation using tropical high-quality timber species in marginal tropical areas is presently practiced. In Xishuangbanna Tropical Botanical Garden (XTBG, 21°54′N, 101°46′E), air temperature usually decreases to 12/25 ℃ at nigh/day during winter. Because a lot of tropical trees showed large decrease in photosynthetic rate in winter in XTBG (Cao *et al.*, 2006; Jiang, 2008), the air temperature in winter in XTBG is regarded as relative low temperature for tropical trees. Therefore, those tropical tree species that are able to survive in the low winter temperature in XTBG should have feasible mechanisms for protecting photosynthetic apparatus from photoinhibition.

Light is the driving force of photosynthesis, but excess light could induce photoinhibition (Barber and Andersson, 1992; Aro *et al.*, 1993; Adir *et al.*, 2005). Photoinhibition is regarded as a decline of photochemistry efficiency under the conditions in which the input of photons exceeds the requirement for photosynthesis (Powles, 1984). Photoinhibition of photosystem II (PSII) is caused probably by two aspects: one is the damage to oxygen-evolving complexes (OEC) (Hakala *et al.*, 2005; Ohnishi *et al.*, 2005; Takahashi *et al.*, 2009; Oguchi *et al.*, 2011; for a review on ROS, see Danon, 2012); the other one is the generation of reactive oxygen species (ROS) which could be induced by excess light excitation (Kornyeyev *et al.*, 2001, 2003a, b; Sainz *et al.*, 2010). Recent studies have indicated that ROS aggravate PSII photoinhibition primarily by inhibiting the repair cycle of PSII photodamage by inhibition of D1 protein synthesis but not by damaging PSII directly (Nishiyama *et al.*, 2001, 2004, 2005, 2006, 2011; Allakhverdiev and Murata, 2004). Low temperature depresses photosynthesis and increases excess light energy, and then increase the risk of PSII photoinhibition. Our previous studies indicated that PSII in tropical trees is very sensitive to a chilling temperature of 4 ℃ associated with moderate light (Huang *et al.*, 2010a, b). However, the mechanism underlying photosynthetic acclimation of tropical tree species to relative winter low temperature of 12/25 ℃ at nigh/day it is unclear.

Plants have the ability to dissipate excitation energy harmlessly as heat in the antenna proteins of PSII (Demmig-Adams, 1990; Asada, 1999; Niyogi, 1999, 2000; Takahashi *et al.*, 2009), which is called qE and is measured as a component of non-photochemical quenching of chlorophyll fluorescence (NPQ). The activation of NPQ is regulated by low lumenal pH, which is accompanied by the generation of proton gradient across the thylakoid membrane (ΔpH) (Gilmore *et al.*, 1998). Cyclic electron flow (CEF) helps the generation of ΔpH, and further favors the activation of NPQ (Munekage *et al.*, 2002, 2004; Takahashi *et al.*, 2009). Furthermore, the activation of NPQ is dependent on xanthophyll cycle. The size of xanthophylls pool is positively correlated the capacity of NPQ. In previous studies on acclimation of photosynthesis to low temperature, it is clearly documented that the up-regulation of NPQ is an important mechanism for affording photoprotection for PSII (Krivosheeva *et al.*, 1996; Verhoeven *et al.*, 1999; Hormaetxe *et al.*, 2004; Ballottari *et al.*, 2007). However, little studies focus on the role of CEF in acclimation of PSII activity to natural low temperature in winter. Previous studies indicated that NPQ was enhanced in tropical trees in

winter in marginal tropical areas (Elsheery *et al.*, 2007; Zhu *et al.*, 2009). We speculate that the enhancement of NPQ in tropical tree species in winter is due to the stimulation of CEF.

When the PSI acceptor side is over-reduced, the recombination between the radical pairs $P700^+/A0^-$ or $P700^+/A1^-$ can generate the triplet state of P700 (Shuvalov *et al.*, 1986; Golbeck, 1987; Golbeck and Bryant, 1991). Chlorophyll triplets can react with molecular oxygen to produce very toxic singlet oxygen that could cause photoinhibitory damage to PSI. Therefore, there are two main mechanisms for the PSI photodamage: the accumulation of hydroxyl radicals at PSI acceptor side and the over-reduction of the PSI acceptor side. It has been reported that CEF is essential for photoprotection of PSI in *Arabidopsis* (Munekage *et al.*, 2002, 2004; Joliot and Johnson, 2011) and tropical tree species (Huang *et al.*, 2011). CEF is regarded as a mechanism that maintains high P700 oxidation ratio and alleviates over-reduction of the acceptor side of PSI (Munekage *et al.*, 2002, 2004, 2008). Oxidized P700 ($P700^+$) can harmlessly quench excess excitation energy as heat and thereby efficiently ameliorating the deleterious effects of excess light (Nuijs *et al.*, 1986). The metal centers in the PSI acceptor side (F_X and F_A/F_B) of *Arabidopsis* mutant plants *pgr5* is over-reduced under high light (Munekage *et al.*, 2008). The over-reduced PSI acceptors react with superoxide, generating hydroxyl radicals which damage PSI reaction centers. Previous studies indicated that PSI is insusceptible to chilling and light stress in tropical trees grown under high light (Huang *et al.*, 2010a, b). However, the response of PSI activity in tropical trees to natural low winter temperature in marginal tropical areas is unknown.

1 Materials and methods

1.1 Plant materials and growth conditions

The following two tropical evergreen tree species were chosen for the study. *Erythrophleum guineense* G. Don (Fabaceae) is a large canopy species native to west coast of Africa and mainly distribute in Guinea and Senegambia. *Dalbergia odorifera* T. Chen (Fabaceae) is native to the Hainan island of China (yearly average air temperature 23 – 25 ℃) and a light-demanding tree species that inhabits secondary forests. These two species produce high-quality timber and their adult plants exhibit good growth performance in the Xishuangbanna Tropical Botanical Garden (XTBG) (21°54′N, 101°46′E) that is located in the northern boundary of the tropical zone. Because these two evergreen species do not drop their leaves in winter, we used them to study the effect of natural winter low temperature on CEF and PSI activity. Three years older seedlings of these two species were used in this study. During the experiment, none of the plants experienced any water or nutrient stresses. At least five mature sun leaves that had flushed in May 2010 were chosen for measuring in situ photosynthetic parameters in both summer and winter.

We mainly conducted photosynthetic measurements in July 2010 (summer) and January 2011 (winter). In winter, the outdoor air temperatures at night and noon are ≈12 ℃ and ≈25 ℃, respectively. In summer, the outdoor air temperatures at night and noon are ≈22 ℃ and ≈35 ℃, respectively. The highest photosynthetic photon flux density (PPFD) at midday is up to 1 850 μmol photons m^{-2} s^{-1} in summer and 1 350 μmol photons m^{-2} s^{-1} in winter.

1.2 Leaf pigment composition measurements

The contents for carotenoids and chlorophylls *a* and *b* for leaves of *E. guineense* and *D. odorifera* in summer and winter were determined according to the method of Lichtenthaler and Wellburn (1983).

1.3 Photoinhibitory treatment

To examine the role of CEF in photoprotection for PSII in leaves of *E. guineense* and *D. odorifera* grown in an open field, detached leaves were vacuum incubated with H_2O or antimycin A (AA, 10 μM, to specifically inhibit PGR5-dependent CEF) in darkness and then treated with 25 ℃ and 1 000 μmol photons m^{-2} s^{-1} for 2 hours on water. These photoinhibitory treatments were conducted in 6 Sep-

tember in 2012.

1.4 Chlorophyll fluorescence and P700 measurements

To analyze the changes in distribution of light energy in PSII and P700 redox state after full acclimation to winter, in winter and summer, we conducted measurements for the light responses of chlorophyll fluorescence and P700 redox state synchronously in detached leaves at a controlled temperature of 25 ℃ with the Dual PAM-100 (Heinz Walz, Effeltrich, Germany) connected to a computer with control software. Six to eight mature leaves were light-adapted (450 μmol photons m^{-2} s^{-1}) for 20 min at 25 ℃ before the measurement of light response curves, and light-adapted fluorescence parameters were recorded after 2 min exposure to each light intensity.

Chlorophyll fluorescence measurements were used to calculate the following parameters: $F_v/F_m=(F_m-F_o)/F_m$, $F_o'=F_o/(F_v/F_m+F_o/F_m')$ (Oxborough and Baker, 1997), $F_v'/F_m'=(F_m'-F_o')/F_m'$; qL $=(F_m'-F_s)/(F_m'-F_o')\times F_o'/F_s$ (Kramer *et al.*, 2004), Y(II) $=(F_m'-F_s)/F_m'$ (Genty *et al.*, 1989), Y(NO) $=F_s/F_m$, Y(NPQ) $=F_s/F_m'-F_s/F_m$, (Hendrickson *et al.*, 2004; Kramer *et al.*, 2004). F_o and F_o' are the minimum fluorescence values in the dark-adapted state and light-adapted state, respectively. F_m and F_m' are the dark-adapted and light-adapted maximum fluorescence upon illumination with a pulse (300 ms) of saturating light (10 000 μmol photons m^{-2} s^{-1}), respectively. F_o and F_m were determined after an overnight dark adaptation. F_s is steady-state fluorescence in light. The ratio F_v/F_m, where $F_v=(F_m-F_o)$ is the variable fluorescence, reflects denotes the maximum quantum yield of PSII (see e.g., Govindjee, 2004); was measured after an overnight dark adaptation. F_v'/F_m' is the maximum quantum yield of PSII after light adaptation. qL is the fraction of open PSII centers. Y(II) is the effective quantum yield of PSII. Y(NO) is the quantum yield of non-regulated energy dissipation of PSII. Y(NPQ) is the quantum yield of regulated energy dissipation of PSII, mainly through the xanthophyll cycle.

The P700 redox state was measured by Dual PAM-100 with a dual wavelength (830/875 nm) unit (Klughammer and Schreiber, 1994), which was widely used in recent studies (Huang *et al.*, 2010b, 2011, 2012a, b, 2013; Yamori *et al.*, 2011). Saturation pulses (10 000 μmol photons m^{-2} s^{-1}), which were introduced primarily for PAM fluorescence measurement, were applied for the assessment of P700 parameters as well. The $P700^+$ signals (P) may vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). P_m was determined after a saturation pulse was given after 10 seconds pre-illumination with far-red light. The value of P_m is calculated from the difference between maximal and the baseline and by the software of Dual-PAM-100. At a defined optical property, the amplitude of P_m depends on the maximum amount of photo-oxidizable P700, which is a parameter for representing the quantity of efficient PSI complex (Zhang and Scheller, 2004; Huang *et al.*, 2010a, b). P_m' was also defined in analogy to the fluorescence parameter F_m' as P_m'; it was determined similarly to P_m, but with background actinic light instead of far-red illumination.

The photochemical quantum yield of PSI, Y(I), is defined by the fraction of overall P700 that in a given state is reduced and not limited by the acceptor side. It is calculated as Y(I) $=(P_m'-P)/P_m$. Y(ND), represents the fraction of overall P700 that is oxidized in a given state, which is enhanced by a trans-thylakoid proton gradient (photosynthetic control at cytb/f complex as well as down-regulation of PSII) and photodamage to PSII. Y(ND) $=P/P_m$. Y(NA); thus, it represents the fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of oxidized acceptors. Y(NA) $=(P_m-P_m')/P_m$. We note that Y(I)+Y(ND)+Y(NA) $=1$.

1.5 Statistical analysis

The results were displayed as mean values of

five independent experiments. The data were subjected to analysis of variance (ANOVA) using the SPSS 16.0 statistical software. Tukey's multiple comparison test was used at $\alpha = 0.05$ significance level to determine whether significant differences existed between different treatments.

2 Results

The maximum quantum yield of PSII (F_v/F_m) and maximum photo-oxidizable P700 (P_m) did not significantly change in *Erythrophleum guineense* and *Dalbergia odorifera* after full acclimation to winter in this northern tropical area (Fig. 1A, B). Because F_v/F_m and P_m represents PSII activity and PSI activity, respectively, this result indicated that PSI and PSII activities of *E. guineense* and *D. odorifera* was protected during the acclimation to low temperature in winter in a marginal tropical area. The chlorophyll (Chl) a and b content and carotenoid content did not differ significantly between summer and winter for both species (Fig. 1C, D), indicating the stability of photosynthetic apparatus of *E. guineense* and *D. odorifera* in winter in the marginal tropical area.

Light response curves indicated that qL largely decreased in winter compared with summer, and then led to the strong decrease in Y(II) in *E. guineense* and *D. odorifera* (Fig. 2A, B), indicating the ability of tropical trees to utilize the products of linear electron flow (LEF) was severely inhibited. Meanwhile, the fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism [Y(NPQ)] strongly increased in winter in the two species (Fig. 2C). The fraction of energy that is passively dissipated in form of heat and fluorescence [Y(NO)] changed slightly in winter compared with summer (Fig. 2D), indicating that excess light energy was harmlessly dissipated in winter.

Because Y(II) is responsible for LEF and Y(I) involves LEF and CEF, if CEF is activated, the value of Y(I)/Y(II) will increase (Yamori *et al.*, 2011; Huang *et al.*, 2012b; 2013). As a result, the increase in Y(I)/Y(II) ratio has been regarded as an indicator of the activation of CEF (Yamori *et al.*, 2011; Huang *et al.*, 2013). In our present study, the Y(I)/Y(II) ratio highly increased in winter compared with summer (Fig. 3). In summer the value of Y(I)/Y(II) under a light of 834 μmol photons m^{-2} s^{-1} was 1.4 in *E. guineense* and 1.3 in *D. odorifera*. In winter, the value of Y(I)/Y(II) under a light of

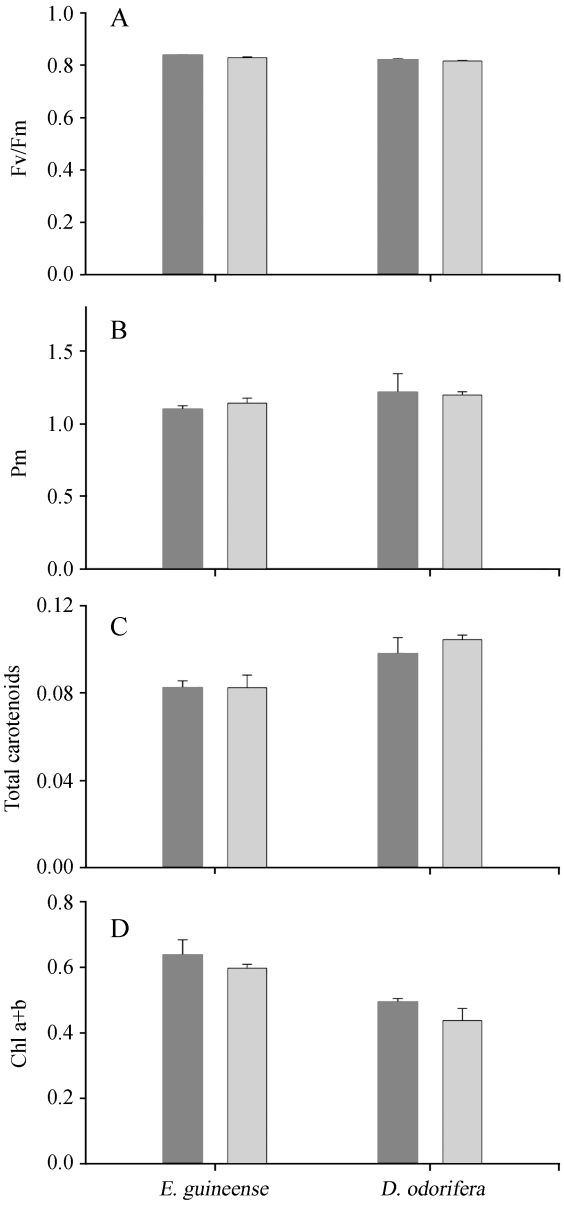

Fig. 1 The maximum quantum yield of PSII (F_v/F_m), maximum photo-oxidizable P700 (P_m) and pigment concentration (g m^{-2}) for leaves of *E. guineense* and *D. odorifera* in summer (*black bars*) and winter (*grey bars*). The mean ± SE was calculated from five independent plants

834 μmol photons m^{-2} s^{-1} increased to 2.6 in *E. guineense* and 2.3 in *D. odorifera*. These results indicated the stimulation of CEF in winter compared with summer in the two tropical tree species.

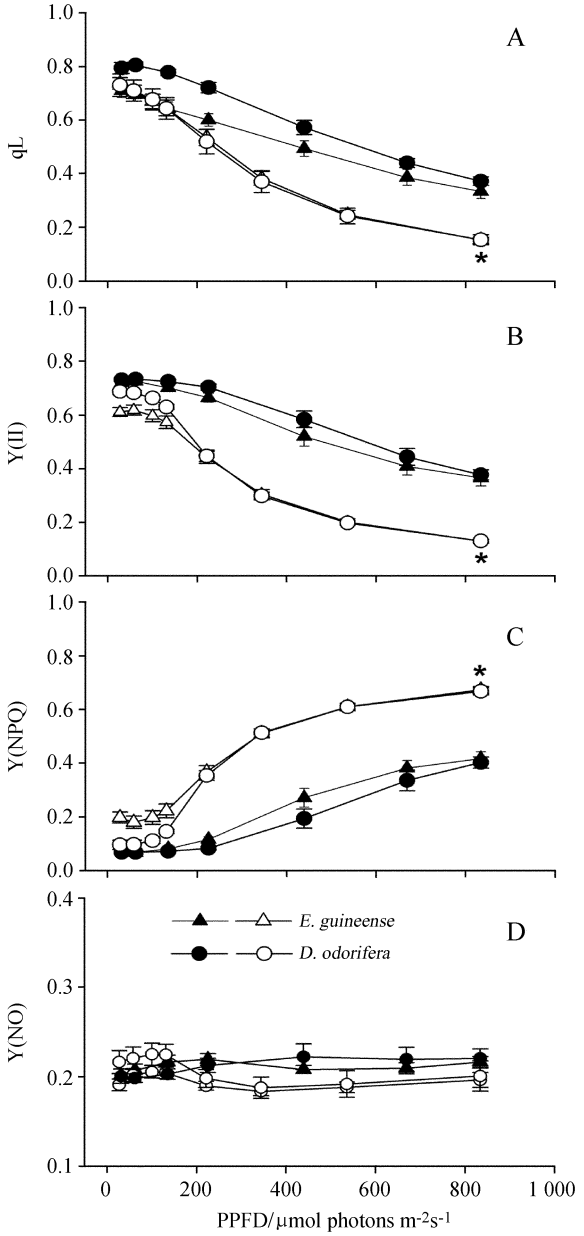

Fig. 2　Light response change in distribution of light energy in PSII for leaves of *E. guineense* and *D. odorifera* in summer (*closed symbols*) and winter (*open symbols*). The mean ± *SE* was calculated from six independent plants. qL-fraction of open PSII centers; Y(II)-effective quantum yield of photosystem II; Y(NPQ)-fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism; Y(NO)-fraction of energy that is passively dissipated in form of heat and fluorescence. Asterisk (*) represents significant difference between summer and winter

The quantum yield of PSI [Y(I)] under high light significantly decreased in winter compared with summer in both species (Fig. 4A). The fraction of total P700 that is oxidized in a given state [Y(ND)] increased in winter compared with summer in both species (Fig. 4B). In winter, the fraction of total P700 that cannot be oxidized in a given state [Y(NA)] was maintained lower than 0.1 (Fig. 4C), suggesting the over-reduction of acceptor in PSI was prevented in winter in both species. Since the activation of CEF is necessary for the high oxidation ratio of P700 and low acceptor side limitation of PSI, the high Y(ND) and a low Y(NA) implied that CEF was stimulated in winter.

Pooling the data of Y(I)/Y(II) ratio and Y(NPQ) in the two species measured at summer and winter, the value of Y(NPQ) was significantly and linearly correlated with the value of Y(I)/Y(II) ratio (Fig. 5), indicating that the stimulation of NPQ in both species in winter was significantly correlated with the up-regulation of CEF.

To examine the role of CEF in protecting PSII from photoinhibition under high light, leaves of *E. guineense* and *D. odorifera* were vacuum infiltrated with antimycin A (AA, a specific inhibitor for PGR5-dependent CEF) solution and then illuminated under high light of 1 000 μmol photons m^{-2} s^{-1} at 25 ℃ for

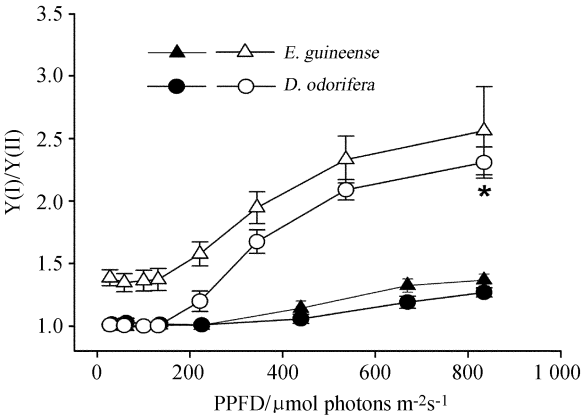

Fig. 3　Light response change in Y(I)/Y(II) for leaves of *E. guineense* and *D. odorifera* in summer (*closed symbols*) and winter (*open symbols*). The mean ± SE was calculated from six independent plants. Asterisk (*) represents significant difference between summer and winter

2 hours. After the high light treatment, the maximum quantum yield of PSII after dark-adaptation (F_v/F_m) in the AA-treated samples decreased to 0.41 in *E. guineense* and 0.49 in *D. odorifera* (Fig. 6). In the H_2O-treated samples, F_v/F_m decreased to 0.61 in *E. guineense* and 0.65 in *D. odorifera* (Fig. 6). The decrease in F_v/F_m in the AA-treated samples was significantly larger than that in the H_2O-treated leaves in both species, indicating that PGR5-dependent CEF plays an important role in protecting PSII for leaves of these two evergreen tropical tree species.

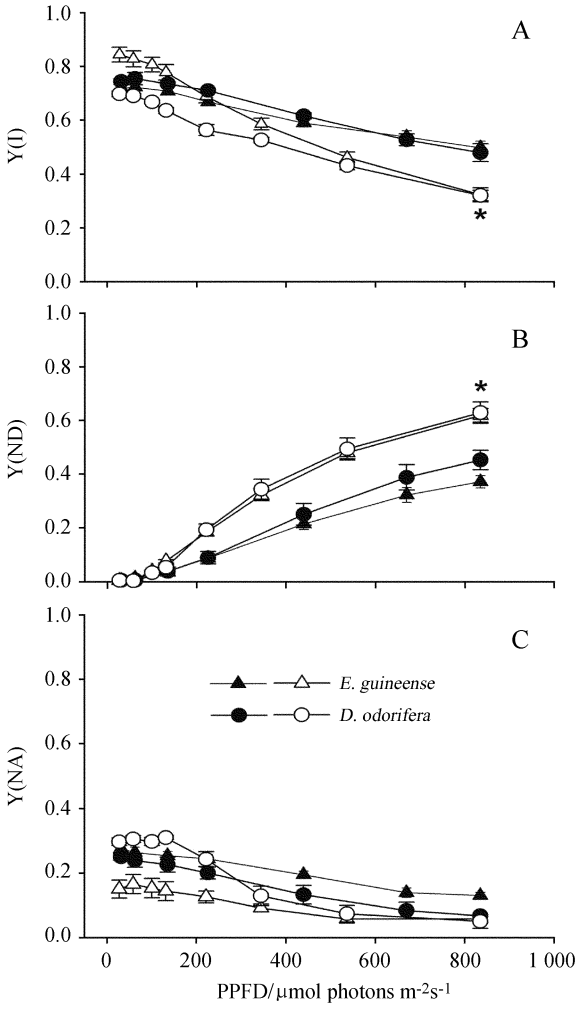

Fig. 4 Light response change in Y(I), Y(ND) and Y(NA) for leaves of *E. guineense* and *D. odorifera* in summer (*closed symbols*) and winter (*open symbols*). The mean ± SE was calculated from six independent plants. Y(I)–effective quantum yield of photosystem I; Y(ND)–fraction of overall P700 that is oxidized in a given state; Y(NA)–fraction of overall P700 that cannot be oxidized in a given state. Asterisk (*) represents significant difference between summer and winter

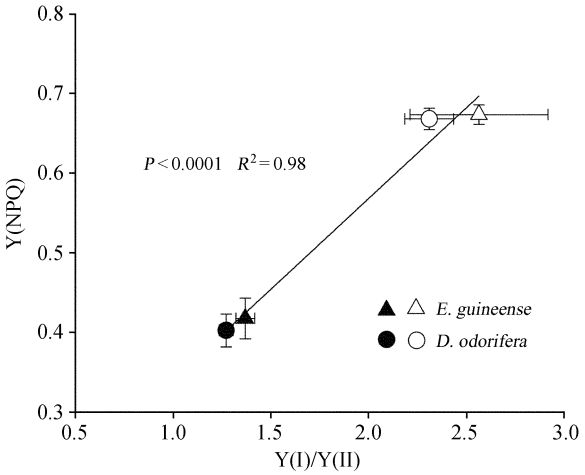

Fig. 5 Change in Y(NPQ) as a function of Y(I)/Y(II) ratio measured at 25 ℃ and 834 μmol photons m^{-2} s^{-1} in summer (*closed symbols*) and winter (*open symbols*) for leaves of *E. guineense* and *D. odorifera*

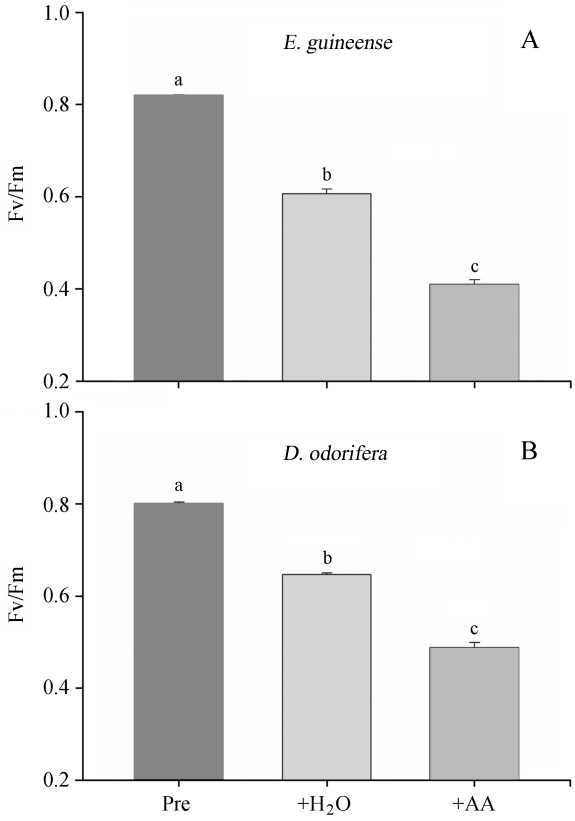

Fig. 6 Effect of antimycin A (AA) on PSII photoinhibition in leaves of *E. guineense* (A) and *D. odorifera* (B). After infiltration as described in Materials and Methods, detached leaves were exposed to light at 1 000 μmol photons m^{-2} s^{-1} at 25 ℃ for 2 hours. The maximum quantum yield of PSII (F_v/F_m) was measured after 30 min dark adaptation. The mean ± SE was calculated from six independent experiments. Different letters (a, b, c) indicate a significant difference between different treatments (One-Way ANOVA, $P<0.05$).

3 Discussion

3.1 Stability of photosynthetic apparatus after full winter acclimation

Although air temperature decreased by 10 ℃ in winter compared with summer and to ~12 ℃ at night and ~25 ℃ at noon at the study site, *E. guineense* and *D. odorifera* maintained stable maximum quantum yield of PSII (F_v/F_m), maximum photo-oxidizable P700 (P_m) and pigment concentrations in winter compared to summer (Fig. 1), indicating the stability of photosynthetic apparatus after full winter acclimation in the both species. Night chilling temperature could inhibit the photosynthetic capacity and induce photoinhibition of PSII in the daytime (Flexas *et al.*, 1999; Allen *et al.*, 2000). Both studied species showed largely decreases in qL and Y(II) in winter (Fig. 2A, B), suggesting that night low temperature in winter depressed the ability to utilize light energy in the daytime in tropical tree species. Both studied species showed sensitivity of PSII activity to chilling temperature associated with light stress (Huang *et al.*, 2010a, b). The stability of PSII activity in winter in both species (Fig. 1A) suggested that these two species have effective mechanisms to protect PSII against night low temperature in winter in the marginal tropical area. Previous studies have indicated that PSI activity is sensitive to chilling temperature in *Arabidopsis thaliana* and cucumber (Sonoike, 1996, 2006; Zhang and Scheller, 2004). Our previous study indicated that PSI was sensitive to chilling temperature under moderate light intensity in *E. guineense* (Huang *et al.*, 2010a). The stability of PSI activity in *E. guineense* in winter (Fig. 1B) suggested that protective mechanisms for PSI were activated in *E. guineense* during acclimation to winter low temperature.

3.2 Stimulation of cyclic electron flow in winter favors photoprotection for PSII

Our results indicated that CEF was significantly stimulated in winter in the two tropical tree species (Fig. 3), which was accompanied with high NPQ (Fig. 2C). Previous studies (Guo and Cao, 2004; Feng and Cao, 2005; Elsheery *et al.*, 2007; Jiang, 2008; Zhu *et al.*, 2009) and our present results indicated that NPQ is significantly stimulated in tropical trees by low winter temperature in the marginal tropical area. We found that the stimulation of CEF was significantly and positively correlated with the up-regulation of NPQ in winter in both species (Fig. 5). Because the activation of NPQ is dependent on the formation of proton gradient across thylakoid membrane that is largely based on cyclic electron flow (Munekage *et al.*, 2002, 2004; Takahashi *et al.*, 2009; Johnson, 2011), these results indicated that the strong up-regulation of NPQ during acclimation to winter low temperature is largely attributable to the stimulation of CEF.

In winter, the decrease in air temperature induced the depression in Y(II). To maintain the stability of PSII activity, plants would up-regulate NPQ to harmlessly dissipate excess light energy and then alleviate the production of ROS that inhibit the repair of PSII activity. It is well documented that PGR5-dependent CEF is essential for activation of NPQ in *Arabidopsis thaliana* (Munakage *et al.*, 2002, 2004; Takahashi *et al.*, 2009). Antimycin A specifically inhibits PGR5-dependent CEF (Munekage *et al.*, 2002; Shikanai, 2007). After high light treatment, the AA-treated samples displayed more PSII photoinhibition than the H_2O-treated samples in the two studied tree species (Fig. 6), suggesting that PGR5-dependent CEF is essential for alleviating PSII photoinhibition in tropical evergreen tree species.

3.3 Stimulation of cyclic electron flow in winter favors photoprotection for PSI

In the chilling-sensitive herbaceous plant cucumber, the preferential damage to photosystem I (PSI) was suggested to be caused by the oxidation by hydroxyl radicals (Sonoike, 1996, 2006). The hydroxyl radicals, the most reactive species of active oxygen, which are generated by the reaction between hydrogen peroxide and photoreduced iron-sulfur centers, destroy PSI at the site of production of hydroxyl radicals (Sonoike, 2006, 2011). Our results indi-

cated that the value of Y(ND) was much higher in winter than in summer in both species (Fig. 4B). $P700^+$ can dissipate excess light energy harmlessly as heat (Nuijs *et al.*, 1986). Furthermore, Y(NA) was low under high light in winter after full winter acclimation (Fig. 4C), suggesting the over-reduction of PSI acceptor side was prevented. In *Arabidopsis thaliana*, the stoichiometry of any Lhca (light-harvesting complex a) antenna proteins with respect to PSI core complex did not change during acclimation to low temperature (Ballotarri *et al.*, 2007). However, the underlying mechanism of how overwintering plants prevent PSI photoinhibition during acclimation to winter low temperature is unclear. Our present study indicated that the stability of PSI activity during acclimation to winter low temperature in both tropical tree species involves the stimulation of CEF. The stimulation of CEF in winter leads to a high level of Y(ND) and a low level of Y(NA) in both species, suggesting that stimulation of CEF is an important mechanism for the stability of PSI activity during the acclimation of overwintering plants to natural low temperature in winter.

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